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**WO 03/105828 A1**

(54) Title: COMBINATION OF EPOTHILONE ANALOGS AND CHEMOTHERAPEUTIC AGENTS FOR THE TREATMENT OF PROLIFERATIVE DISEASES

(57) Abstract: Compositions and methods are disclosed which are useful of the treatment and prevention of proliferative diseases.

**COMBINATION OF EPOTHILONE ANALOGS AND  
CHEMOTHERAPEUTIC AGENTS FOR THE TREATMENT OF  
PROLIFERATIVE DISEASES**

5                   **CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims priority from provisional U. S. Application Serial Number 60/388,702, filed on June 14, 2002, incorporated herein by reference in its entirety.

10                   **FIELD OF THE INVENTION**

This invention relates to the fields of oncology and improved chemotherapy regimens.

**BACKGROUND OF THE INVENTION**

15                   The disclosure of each literature article and published patent document referred to herein is incorporated by reference in its entirety.

The National Cancer Institute has estimated that in the United States alone, 1 in 3 people will be struck with cancer during their lifetime. Moreover, approximately 50% to 60% of people contracting cancer will eventually succumb to the disease. The  
20                   widespread occurrence of this disease underscores the need for improved anticancer regimens for the treatment of malignancy.

Due to the wide variety of cancers presently observed, numerous anticancer agents have been developed to destroy cancer within the body. These compounds are administered to cancer patients with the objective of destroying or otherwise  
25                   inhibiting the growth of malignant cells while leaving normal, healthy cells undisturbed. Anticancer agents have been classified based upon their mechanism of action.

One type of chemotherapeutic is referred to as a metal coordination complex. It is believed this type of chemotherapeutic forms predominantly inter-strand DNA  
30                   cross links in the nuclei of cells, thereby preventing cellular replication. As a result, tumor growth is initially repressed, and then reversed. Another type of chemotherapeutic is referred to as an alkylating agent. These compounds function by

inserting foreign compositions or molecules into the DNA of dividing cancer cells. As a result of these foreign moieties, the normal functions of cancer cells are disrupted and proliferation is prevented. Another type of chemotherapeutic is an antineoplastic agent. This type of agent prevents, kills, or blocks the growth and spread of cancer  
5 cells. Still other types of anticancer agents include nonsteroidal aromatase inhibitors, bifunctional alkylating agents, etc.

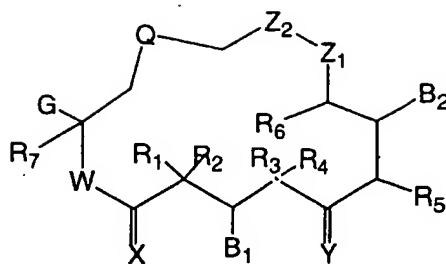
Paclitaxel represents one of the major classes of antimicrotubule agents that promotes tubulin polymerization and, presumably, mitotic arrest during cell division. Taxol (paclitaxel) has been shown to have excellent antitumor activity *in vivo* and has  
10 been employed in the treatment of a variety of cancers, including breast, ovarian and lung cancer. Unfortunately, many tumors develop resistance to paclitaxel.

Epothilones mimic the biological effects of taxol, (Bollag et al., Cancer Research 55: 2325-2333 (1995), and in competition studies act as competitive inhibitors of taxol binding to microtubules. However, epothilones enjoy a significant  
15 advantage over taxol in that epothilones exhibit a much lower drop in potency compared to taxol against a multiple drug-resistant cell line (Bollag et al. (1995)). Furthermore, epothilones are considerably less efficiently exported from the cells by P-glycoprotein than is taxol (Gerth et al. (1996)).

It is an object of the invention to provide efficacious combination  
20 chemotherapeutic treatment regimens wherein epothilone analogs are combined with other anti-neoplastic agents for the treatment of proliferative diseases.

### SUMMARY OF THE INVENTION

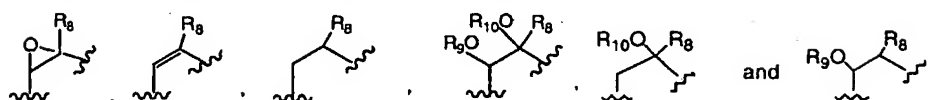
The present invention provides a method for the treatment of anti-proliferative  
25 diseases, including cancer, which comprises administering to a mammalian specie in need thereof, a therapeutically effective amount of: (1) a compound of formula I and (2) at least one anti-proliferative agent.



(I)

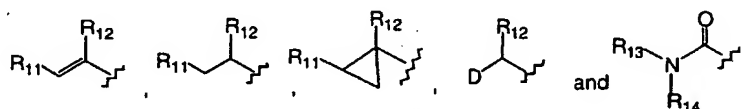
wherein:

5 Q is selected from the group consisting of



G is selected from the group consisting of alkyl, substituted alkyl, aryl,  
substituted aryl, heterocyclo,

10



W is O or N R<sub>15</sub>;

X is O or H, H;

Y is selected from the group consisting of O; H, OR<sub>16</sub>; OR<sub>17</sub>; NOR<sub>18</sub>; H,  
15 NHOR<sub>19</sub>; H, NR<sub>20</sub>R<sub>21</sub>; H, H; and CHR<sub>22</sub>; wherein OR<sub>17</sub>, OR<sub>17</sub> can be a cyclic ketal;

Z<sub>1</sub> and Z<sub>2</sub> are independently selected from the group consisting of CH<sub>2</sub>, O,  
NR<sub>23</sub>, S, and SO<sub>2</sub>, wherein only one of Z<sub>1</sub> and Z<sub>2</sub> can be a heteroatom;

B<sub>1</sub> and B<sub>2</sub> are independently selected from the group consisting of OR<sub>24</sub>,  
OCOR<sub>25</sub>, and O-C(=O)-NR<sub>26</sub>R<sub>27</sub>, and when B<sub>1</sub> is H and Y is OH, H, they can form a  
20 six-membered ring ketal or acetal;

D is selected from the group consisting of NR<sub>28</sub>R<sub>29</sub>, NR<sub>30</sub>COR<sub>31</sub> and saturated  
heterocycle;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>13</sub>, R<sub>14</sub>, R<sub>18</sub>, R<sub>19</sub>, R<sub>20</sub>, R<sub>21</sub>, R<sub>22</sub>, R<sub>26</sub> and R<sub>27</sub> are  
independently selected from the group consisting of H, alkyl, substituted alkyl, and  
25 aryl, and when R<sub>1</sub> and R<sub>2</sub> are alkyl can be joined to form a cycloalkyl, and when R<sub>3</sub>

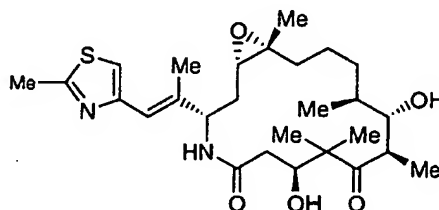
and R<sub>4</sub> are alkyl can be joined to form a cycloalkyl;

R<sub>9</sub>, R<sub>10</sub>, R<sub>16</sub>, R<sub>17</sub>, R<sub>24</sub>, R<sub>25</sub> and R<sub>31</sub> are independently selected from the group consisting of H, alkyl, and substituted alkyl;

R<sub>8</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>28</sub>, R<sub>30</sub>, R<sub>32</sub>, and R<sub>33</sub> are independently selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl and heterocyclo;

R<sub>15</sub>, R<sub>23</sub> and R<sub>29</sub> are independently selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo, R<sub>32</sub>C=O, R<sub>33</sub>SO<sub>2</sub>, hydroxy, O-alkyl or O-substituted alkyl; and  
pharmaceutically acceptable salts thereof and any hydrates, solvates or geometric, optical and stereoisomers thereof.

An example of a compound of Formula I is the compound of Formula Ia ("Compound 1"):



Ia

Suitable anti-proliferative agents for use in the methods of the invention, include, without limitation, alkylating agents (including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): Uracil mustard, Chlormethine, Cyclophosphamide (Cytosan), Carboplatin, Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylene-melamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, and Temozolomide; antimetabolites (including, without limitation, folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors), Methotrexate, 5-Fluorouracil, Floxuridine, Cytarabine, 6-Mercaptopurine,

6-Thioguanine, Fludarabine phosphate, Pentostatine, and Gemcitabine; natural products and their derivatives (for example, vinca alkaloids, antitumor antibiotics, enzymes, lymphokines and epipodophyllotoxins): Vinblastine, Vincristine, Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Ara-C, 5 paclitaxel (paclitaxel is commercially available as Taxol®), Mithramycin, Deoxycoformycin, Mitomycin-C, L-Asparaginase, Interferons (especially IFN- $\alpha$ ), Etoposide, and Teniposide; navelbene, CPT-11; anastrozole, letrozole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine and radiation.

According to the present invention, the antiproliferative agent is administered 10 prior to, simultaneously with, or after the administration of a compound of Formula I, such as the compound of Formula Ia.

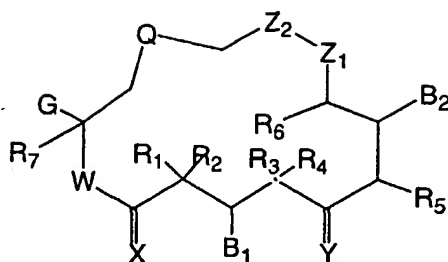
### DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, methods for the scheduled 15 administration of epothilone analogs in combination(s) with at least one additional anti-neoplastic agent for the treatment and prevention of proliferative diseases are provided.

Epothilones mimic the biological effects of taxol, (Bollag et al., Cancer Research 55: 2325-2333 (1995), and in competition studies act as competitive 20 inhibitors of taxol binding to microtubules. However, epothilones enjoy a significant advantage over taxol in that epothilones exhibit a much lower drop in potency compared to taxol against a multiple drug-resistant cell line (Bollag et al. (1995)). Furthermore, epothilones are considerably less efficiently exported from the cells by P-glycoprotein than is taxol (Gerth et al. (1996)).

25 In one embodiment of the present invention, the chemotherapeutic method of the invention comprises the administration of an epothilone analog of Formula I in combination with at least one other anti-cancer or anti-proliferative agent. The epothilone analogs disclosed herein, when used in combination with at least one other anti-cancer agent, demonstrate superior cytotoxic activity.

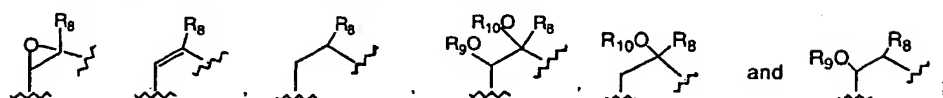
30 Epothilone analogs for use in the methods of the invention are compounds of Formula I:



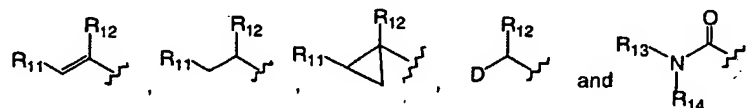
I

5 wherein:

Q is selected from the group consisting of



G is selected from the group consisting of alkyl, substituted alkyl, aryl,  
 10 substituted aryl, heterocyclo,



W is O or N R<sub>15</sub>;

X is O or H, H;

15 Y is selected from the group consisting of O; H, OR<sub>16</sub>; OR<sub>17</sub>, OR<sub>17</sub>; NOR<sub>18</sub>; H, NHOR<sub>19</sub>; H, NR<sub>20</sub>R<sub>21</sub>; H, H; and CHR<sub>22</sub>; wherein OR<sub>17</sub>, OR<sub>17</sub> can be a cyclic ketal;

Z<sub>1</sub> and Z<sub>2</sub> are independently selected from the group consisting of CH<sub>2</sub>, O, NR<sub>23</sub>, S, and SO<sub>2</sub>, wherein only one of Z<sub>1</sub> and Z<sub>2</sub> can be a heteroatom;

B<sub>1</sub> and B<sub>2</sub> are independently selected from the group consisting of OR<sub>24</sub>,  
 20 OCOR<sub>25</sub>, and O-C(=O)-NR<sub>26</sub>R<sub>27</sub>, and when B<sub>1</sub> is H and Y is OH, H, they can form a six-membered ring ketal or acetal;

D is selected from the group consisting of NR<sub>28</sub>R<sub>29</sub>, NR<sub>30</sub>COR<sub>31</sub> and saturated heterocycle;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>13</sub>, R<sub>14</sub>, R<sub>18</sub>, R<sub>19</sub>, R<sub>20</sub>, R<sub>21</sub>, R<sub>22</sub>, R<sub>26</sub> and R<sub>27</sub> are  
 25 independently selected from the group consisting of H, alkyl, substituted alkyl, and

aryl, and when R<sub>1</sub> and R<sub>2</sub> are alkyl can be joined to form a cycloalkyl, and when R<sub>3</sub> and R<sub>4</sub> are alkyl can be joined to form a cycloalkyl;

R<sub>9</sub>, R<sub>10</sub>, R<sub>16</sub>, R<sub>17</sub>, R<sub>24</sub>, R<sub>25</sub> and R<sub>31</sub> are independently selected from the group consisting of H, alkyl, and substituted alkyl;

5 R<sub>8</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>28</sub>, R<sub>30</sub>, R<sub>32</sub>, and R<sub>33</sub> are independently selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl and heterocyclo;

R<sub>15</sub>, R<sub>23</sub> and R<sub>29</sub> are independently selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo, R<sub>32</sub>C=O,

10 R<sub>33</sub>SO<sub>2</sub>, hydroxy, O-alkyl or O-substituted alkyl; and

pharmaceutically acceptable salts thereof and any hydrates, solvates or geometric, optical and stereoisomers thereof.

According to the present invention, the anti-proliferative agent may be administered prior to, simultaneously with, or after the administration of an epothilone

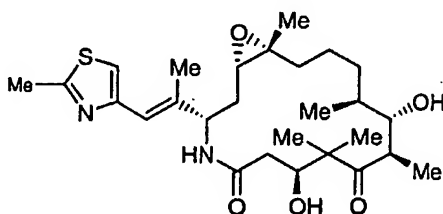
15 analog of Formula I.

Examples of compounds of Formula I include

[1S 1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*, 16S\*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-

17oxabicyclo[14.1.0]-heptadecane-5,9-dione (Formula Ia; Compound 1) and

20 pharmaceutically acceptable salts thereof. Compound 1 has the following chemical structure:



Ia

25

When describing the compounds of the present invention, the phrase "lower alkyl" or "lower alk" (as part of another group) refers to an unsubstituted alkyl group of 1 to 6, such as 1 to 4, carbon atoms.



The term "aralkyl" refers to an aryl group bonded directly through a lower alkyl group. An example of an aralkyl group is benzyl.

The term "aryl" refers to a monocyclic or bicyclic aromatic hydrocarbon group having 6 to 12 carbon atoms in the ring portion. Exemplary of aryl herein are phenyl,  
5 naphthyl and biphenyl groups.

The term "heterocyclo" refers to a fully saturated or unsaturated, aromatic or nonaromatic cyclic group which is a 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 15 membered tricyclic ring system which has at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group  
10 containing a heteroatom may have 1, 2, 3 or 4 heteroatoms selected from nitrogen, oxygen and sulfur where the nitrogen and sulfur heteroatoms may also optionally be oxidized and the nitrogen heteroatoms may also optionally be quaternized. The heterocyclo group may be attached at any heteroatom or carbon atom.

Exemplary monocyclic heterocyclo groups include pyrrolidinyl, pyrrolyl,  
15 indolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxazepinyl, azepinyl, 4-piperidonyl, pyridyl, N-oxo-pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl,  
20 tetrahydrothiopyranyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, tetrahydrothiopyranylsulfone, thiamorpholinyl sulfone, 1,3-dioxolane, tetrahydro-1,1-dioxothienyl, dioxanyl, isothiazolidinyl, thietanyl, thiiranyl, triazinyl, triazolyl, and the like.

Exemplary bicyclic heterocyclo groups include benzothiazolyl, benzoxazolyl,  
25 benzothienyl, quinolinyl, quinolinyl-N-oxide, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,1-b]pyridinyl or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), benzisothiazolyl,  
30 benzisoxazolyl, benzodiazinyl, benzofurazanyl, benzothiopyranyl, benzotriazolyl, benzpyrazolyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydrobenzopyranyl, indolinyl, isochromanyl,

isoindoliny, naphthyridinyl, phthalazinyl, piperonyl, purinyl, pyridopyridyl, quinazolinyl, tetrahydroquinolinyl, thienofuryl, thienopyridyl, thienothienyl, and the like.

When a group is referred to as being optionally substituted, it may be substituted with one to five, preferably one to three, substituents such as F, Cl, Br, I, trifluoromethyl, trifluoromethoxy, hydroxy, lower alkoxy, cycloalkoxy, heterocycloxy, oxo, lower alkanoyl, aryloxy, lower alkanoyloxy, amino, lower alkylamino, arylamino, aralkylamino, cycloalkylamino, heterocycloamino, disubstituted amines in which the two amino substituents independently are selected from lower alkyl, aryl or aralkyl, lower alkanoylamino, aroylamino, aralkanoylamino, substituted lower alkanoylamino, substituted arylamino, substituted aralkylanoylamino, thiol, lower alkylthio, arylthio, aralkylthio, cycloalkylthio, heterocyclothio, lower alkylthiono, arylthiono, aralkylthiono, lower alkylsulfonyl, arylsulfonyl, aralkylsulfonyl, sulfonamide (e.g.,  $\text{SO}_2\text{NH}_2$ ), substituted sulfonamide, nitro, cyano, carboxy, carbamyl (e.g.,  $\text{CONH}_2$ ), substituted carbamyl (e.g.,  $\text{CONH}$ -lower alkyl,  $\text{CONH}$ -aryl,  $\text{CONH}$ -aralkyl or cases where there are two substituents on the nitrogen independently selected from lower alkyl, aryl or aralkyl), lower alkoxy, carbonyl, aryl, substituted aryl, guanidino, and heterocyclos (e.g., indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like). Where noted above that the substituent is further substituted, it will be substituted with F, Cl, Br, I, optionally substituted lower alkyl, hydroxy, optionally substituted lower alkoxy, optionally substituted aryl, or optionally substituted aralkyl.

All stereoisomers of the Formula I compounds of the instant invention are contemplated, either in admixture or in pure or substantially pure form. The definition of the formula I compounds embraces all possible stereoisomers and their mixtures. The Formula I definitions very particularly embrace the racemic forms and the isolated optical isomers having the specified activity.

An example of an epothilone analog of Formula I for use in the methods of the invention is Compound 1: [1S-[1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0] heptadecane-5,9-dione. Compound 1 is described in WO 99/02514, published January 21, 1999, the entire contents of which are herein

incorporated by reference.

Compound 1, an exemplary epothilone analog of the invention, is a semi-synthetic epothilone analog and has a mode of action analogous to paclitaxel (i.e., microtubule stabilization). However, in preclinical pharmacology studies, Compound 1 has demonstrated significant improvement over paclitaxel in several critical aspects. Compound 1 exhibits a very impressive and broad spectrum of antitumor activity against paclitaxel-sensitive (A2780, HCT116 and LS174T) and, more importantly, as well as paclitaxel-resistant human colon tumors (HCT116/VM46), ovarian carcinoma (Pat-7 and A2780Tax) and breast carcinoma (Pat-21) models. Compound 1 is orally efficacious; the antitumor activity produced after oral administration is comparable to that produced by parenteral administration of the drug. These preclinical efficacy data indicate that Compound 1 demonstrates improved clinical efficacy in TAXOL®-insensitive and sensitive disease types.

As used herein, the phrase "anti-neoplastic agent" is synonymous with "chemotherapeutic agent" and/or "anti-proliferative agent" and refers to compounds that prevent cancer, or hyperproliferative cells, from multiplying. Anti-proliferative agents prevent cancer cells from multiplying by: (1) interfering with the cell's ability to replicate DNA and (2) inducing cell death and/or apoptosis in the cancer cells.

Classes of compounds that may be used as anti-proliferative cytotoxic agents include the following:

Alkylating agents (including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): Uracil mustard, Chlormethine, Cyclophosphamide (Cytosan), Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylene-melamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, and Temozolomide.

Antimetabolites (including, without limitation, folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors): Methotrexate, 5-Fluorouracil, Floxuridine, Cytarabine, 6-Mercaptopurine, 6-Thioguanine, Fludarabine phosphate, Pentostatine, and Gemcitabine.

Natural products and their derivatives (for example, vinca alkaloids, antitumor antibiotics, enzymes, lymphokines and epipodophyllotoxins): Vinblastine, Vincristine, Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin,

Idarubicin, Ara-C, paclitaxel (paclitaxel is commercially available as Taxol®), Mithramycin, Deoxyco-formycin, Mitomycin-C, L-Asparaginase, Interferons (especially IFN- $\alpha$ ), Etoposide, and Teniposide.

Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrozole, 5 letrozole, capecitabine, carboplatin, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

The phrase "radiation therapy" includes, but is not limited to, x-rays or gamma rays which are delivered from either an externally applied source such as a beam or by implantation of small radioactive sources.

10 Microtubule affecting agents interfere with cellular mitosis and are well known in the art for their anti-proliferative cytotoxic activity. Microtubule affecting agents useful in the invention include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolastatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin 15 (NSC 332598), paclitaxel (Taxol®, NSC 125973), Taxol® derivatives (e.g., derivatives (e.g., NSC 608832), thiocolchicine NSC 361792), trityl cysteine (NSC 83265), vinblastine sulfate (NSC 49842), vincristine sulfate (NSC 67574), natural and synthetic epothilones including, but not limited to, epothilone A, epothilone B, epothilone C, epothilone D, desoxyepothilone A, desoxyepothilone B, [1S- 20 [1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17 oxabicyclo[14.1.0]heptadecane-5,9-dione (disclosed in WO 99/02514, published January 21, 1999, entire contents of which are herein incorporated by reference), [1S- [1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]]-3-[2-[2-(aminomethyl)-4-thiazolyl]-1-methylethenyl]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-4-17-dioxabicyclo[14.1.0]- 25 heptadecane-5,9-dione (disclosed in U.S. Patent 6,262,094, issued July 17, 2001, entire contents of which are herein incorporated by reference, and examples 7 and 8 therein), and derivatives thereof; and other microtubule disruptor agents. Additional antineoplastic agents include discodermolide (*see* Service, (1996) *Science*, 274:2009) 30 estramustine, nocodazole, MAP4, and the like. Examples of such agents are also described in the scientific and patent literature, see, e.g., Bulinski (1997) *J. Cell Sci.* 110:3055 3064; Panda (1997) *Proc. Natl. Acad. Sci. USA* 94:10560-10564; Muhlradt

(1997) *Cancer Res.* 57:3344-3346; Nicolaou (1997) *Nature* 387:268-272; Vasquez (1997) *Mol. Biol. Cell.* 8:973-985; Panda (1996) *J. Biol. Chem* 271:29807-29812.

In cases where it is desirable to render aberrantly proliferative cells quiescent in conjunction with or prior to treatment with the chemotherapeutic methods of the invention, hormones and steroids (including synthetic analogs): 17a-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Methylprednisolone, Methyl-testosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine, Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, Zoladex can also be administered to the patient.

Also suitable for use in the combination chemotherapeutic methods of the invention are antiangiogenics such as matrix metalloproteinase inhibitors, and other VEGF inhibitors, such as anti-VEGF antibodies and small molecules such as ZD6474 and SU6668 are also included. Anti- Her2 antibodies from Genetech may also be utilized. A suitable EGFR inhibitor is EKB-569 (an irreversible inhibitor). Also included are Imclone antibody C225 immunospecific for the EGFR, and src inhibitors.

Also suitable for use as an antiproliferative cytostatic agent is Casodex<sup>TM</sup> which renders androgen-dependent carcinomas non-proliferative. Yet another example of a cytostatic agent is the antiestrogen Tamoxifen which inhibits the proliferation or growth of estrogen dependent breast cancer. Inhibitors of the transduction of cellular proliferative signals are cytostatic agents. Examples are epidermal growth factor inhibitors, Her-2 inhibitors, MEK-1 kinase inhibitors, MAPK kinase inhibitors, PI3 inhibitors, Src kinase inhibitors, and PDGF inhibitors.

As mentioned, certain anti-proliferative agents are anti-angiogenic and antivascular agents and, by interrupting blood flow to solid tumors, render cancer cells quiescent by depriving them of nutrition. Castration, which also renders androgen dependent carcinomas non-proliferative, may also be utilized. Starvation by means other than surgical disruption of blood flow is another example of a cytostatic agent. One exemplary class of antivascular cytostatic agents is the combretastatins. Other exemplary cytostatic agents include MET kinase inhibitors, MAP kinase inhibitors, inhibitors of non-receptor and receptor tyrosine kinases, inhibitors of integrin signaling, and inhibitors of insulin-like growth factor receptors.

Thus, the present invention provides methods for the treatment of a variety of cancers including, but not limited to, the following:

carcinoma including that of the bladder (including accelerated and metastatic bladder cancer), breast, colon (including colorectal cancer), kidney, liver,  
5 lung (including small and non-small cell lung cancer and lung adenocarcinoma),  
ovary, prostate, testes, genitourinary tract, lymphatic system, rectum, larynx, pancreas  
(including exocrine pancreatic carcinoma), esophagus, stomach, gall bladder, cervix,  
thyroid, and skin (including squamous cell carcinoma);

hematopoietic tumors of lymphoid lineage including leukemia, acute  
10 lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell  
lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma,  
histiocytic lymphoma, and Burketts lymphoma;

hematopoietic tumors of myeloid lineage including acute and chronic  
myelogenous leukemias, myelodysplastic syndrome, myeloid leukemia, and  
15 promyelocytic leukemia;

tumors of the central and peripheral nervous system including  
astrocytoma, neuroblastoma, glioma, and schwannomas;

tumors of mesenchymal origin including fibrosarcoma,  
rhabdomyosarcoma, and osteosarcoma; and

20 other tumors including melanoma, xenoderma pigmentosum,  
keratoactanthoma, seminoma, thyroid follicular cancer, and teratocarcinoma.

For example, in one embodiment, the invention is used to treat accelerated or metastatic cancers, such as bladder cancer, pancreatic cancer, prostate cancer, non-  
small cell lung cancer, colorectal cancer and breast cancer.

25 In another embodiment of this invention, methods are provided for the  
treatment of cancerous tumors. Advantageously, the method of this invention reduces  
the development of tumors, reduces tumor burden, or produces tumor regression in a  
mammalian host.

Methods for the safe and effective administration of most of these  
30 chemotherapeutic agents are known to those skilled in the art. In addition, their  
administration is described in the standard literature.

For example, the administration of many of the chemotherapeutic agents is

described in the "Physicians' Desk Reference" (PDR), *e.g.*, 1996 edition (Medical Economics Company, Montvale, NJ 07645-1742, USA); the disclosure of which is incorporated herein by reference thereto.

The Formula I compounds may be prepared by the procedures described in  
5 WO 99/02514, published January 21, 1999.

The compounds of Formulas I are useful in various pharmaceutically acceptable salt forms. The term "pharmaceutically acceptable salt" refers to those salt forms which would be apparent to the pharmaceutical chemist, *i.e.*, those which are substantially non-toxic and which provide the desired pharmacokinetic properties,  
10 palatability, absorption, distribution, metabolism or excretion. Other factors, more practical in nature, which are also important in the selection, are cost of the raw materials, ease of crystallization, yield, stability, hygroscopicity and flowability of the resulting bulk drug. Conveniently, pharmaceutical compositions may be prepared from the active ingredients or their pharmaceutically acceptable salts in combination  
15 with pharmaceutically acceptable carriers.

Pharmaceutically acceptable salts of the Formula I compounds which are suitable for use in the methods and compositions of the present invention include, but are not limited to, salts formed with a variety of organic and inorganic acids such as hydrogen chloride, hydroxymethane sulfonic acid, hydrogen bromide,  
20 methanesulfonic acid, sulfuric acid, acetic acid, trifluoroacetic acid, maleic acid, benzenesulfonic acid, toluenesulfonic acid, sulfamic acid, glycolic acid, stearic acid, lactic acid, malic acid, pamoic acid, sulfanilic acid, 2-acetoxybenzoic acid, fumaric acid, toluenesulfonic acid, methanesulfonic acid, ethanedisulfonic acid, oxalic acid, isethonic acid, and include various other pharmaceutically acceptable salts, such as,  
25 *e.g.*, nitrates, phosphates, borates, tartrates, citrates, succinates, benzoates, ascorbates, salicylates, and the like. Cations such as quaternary ammonium ions are contemplated as pharmaceutically acceptable counterions for anionic moieties.

Examples of salts of compounds of Formula I include, for example, hydrochloride salts, methanesulfonic acid salts and trifluoroacetic acid salts. In  
30 addition, pharmaceutically acceptable salts of the Formula I compounds may be formed with alkali metals such as sodium, potassium and lithium; alkaline earth metals such as calcium and magnesium; organic bases such as dicyclohexylamine,

tributylamine, and pyridine; and amino acids such as arginine, lysine and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized by conventional chemical methods. Generally, the salts are prepared by reacting the free base or acid with stoichiometric amounts or with an excess of the  
5 desired salt-forming inorganic or organic acid or base, in a suitable solvent or solvent combination.

The present invention also encompasses a pharmaceutical composition useful in the treatment of cancer, comprising the administration of a therapeutically effective amount of the combinations of this invention, with or without pharmaceutically  
10 acceptable carriers or diluents. The pharmaceutical compositions of this invention comprise an anti-proliferative agent or agents, a formula I compound, and a pharmaceutically acceptable carrier. The methods entail the use of a neoplastic agent in combination with a Formula I compound. The compositions of the present invention may further comprise one or more pharmaceutically acceptable additional  
15 ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like. The antineoplastic agents, Formula I compounds and compositions of the present invention may be administered orally or parenterally including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

20 For oral use, the antineoplastic agents, Formula I compounds and compositions of this invention may be administered, for example, in the form of tablets or capsules, powders, dispersible granules, or cachets, or as aqueous solutions or suspensions. In the case of tablets for oral use, carriers which are commonly used include lactose, corn starch, magnesium carbonate, talc, and sugar, and lubricating  
25 agents such as magnesium stearate are commonly added. For oral administration in capsule form, useful carriers include lactose, corn starch, magnesium carbonate, talc, and sugar. When aqueous suspensions are used for oral administration, emulsifying and/or suspending agents are commonly added.

In addition, sweetening and/or flavoring agents may be added to the oral  
30 compositions. For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient(s) are usually employed, and the pH of the solutions should be suitably adjusted and buffered. For intravenous use, the total



concentration of the solute(s) should be controlled in order to render the preparation isotonic.

For preparing suppositories according to the invention, a low melting wax such as a mixture of fatty acid glycerides or cocoa butter is first melted, and the active  
5 ingredient is dispersed homogeneously in the wax, for example by stirring. The molten homogeneous mixture is then poured into conveniently sized molds and allowed to cool and thereby solidify.

Liquid preparations include solutions, suspensions and emulsions. Such preparations are exemplified by water or water/propylene glycol solutions for  
10 parenteral injection. Liquid preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier, such as an inert compressed gas.

15 Also included are solid preparations which are intended for conversion, shortly before use, to liquid preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

The compounds of Formula I, as well as the anti-neoplastic agents, described herein may also be delivered transdermally. The transdermal compositions can take  
20 the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose.

The combinations of the present invention may also be used in conjunction with other well known therapies that are selected for their particular usefulness against  
25 the condition that is being treated.

If formulated as a fixed dose, the active ingredients of the combination compositions of this invention are employed within the dosage ranges described below. Alternatively, the anti-neoplastic, and Formula I compounds may be administered separately in the dosage ranges described below. In a preferred  
30 embodiment of the present invention, the antineoplastic agent is administered in the dosage range described below following administration of the Formula I compound in the dosage range described below.

Table I sets forth preferred chemotherapeutic combinations and exemplary dosages for use in the methods of the present invention. Where "Compound of Formula I" appears, any of the variations of Formula I set forth herein are contemplated for use in the chemotherapeutic combinations. For example, Compound 1 may be employed.

TABLE 1

	CHEMOTHERAPEUTIC COMBINATION	DOSAGE <u>mg/m<sup>2</sup> (per dose)</u>
10	Compound of Formula I + Cisplatin	0.1-100 mg/m <sup>2</sup> 5-150 mg/m <sup>2</sup>
	Compound of Formula I + Carboplatin	0.1-100 mg/m <sup>2</sup> 5-1000 mg/m <sup>2</sup>
15	Compound of Formula I + Radiation	0.1-100 mg/m <sup>2</sup> 200-8000 cGy
20	Compound of Formula I + CPT-11	0.1-100 mg/m <sup>2</sup> 5-400 mg/m <sup>2</sup>
	Compound of Formula I + Paclitaxel	0.1-100 mg/m <sup>2</sup> 40-250 mg/m <sup>2</sup>
25	Compound of Formula I + Paclitaxel + Carboplatin	0.1-100 mg/m <sup>2</sup> 40-250 mg/m <sup>2</sup> 5-1000 mg/m <sup>2</sup>
30	Compound of Formula I + 5FU and optionally + Leucovorin	0.1-100 mg/m <sup>2</sup> 5-5000 mg/m <sup>2</sup> 5-1000 mg/m <sup>2</sup>

	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ Etoposide	1-500 mg/m <sup>2</sup>
5	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ Gemcitabine	100-3000 mg/m <sup>2</sup>
	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ UFT and optionally	50-800 mg/m <sup>2</sup>
10	+ leucovorin	5-1000 mg/m <sup>2</sup>
	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ Gemcitabine	100-3000 mg/m <sup>2</sup>
15	+ Cisplatin	5-150 mg/m <sup>2</sup>
	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+UFT	50-800 mg/m <sup>2</sup>
	+Leucovorin	5-1000 mg/m <sup>2</sup>
20	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ Cisplatin	5-150 mg/m <sup>2</sup>
	+ paclitaxel	40-250 mg/m <sup>2</sup>
	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
25	+ Cisplatin	5-150 mg/m <sup>2</sup>
	+ 5FU	5-5000 mg/m <sup>2</sup>
	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ Oxaliplatin	5-200 mg/m <sup>2</sup>
30	+ CPT-11	4-400 mg/m <sup>2</sup>
	Compound of Formula I	0.1-100 mg/m <sup>2</sup>

	+ 5FU	5-5000 mg/m <sup>2</sup>
	+ CPT-11 and optionally	4-400 mg/m <sup>2</sup>
	+ leucovorin	5-1000 mg/m <sup>2</sup>
5	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ 5FU	5-5000 mg/m <sup>2</sup>
	+ radiation	200-8000 cGy
10	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ radiation	200-8000 cGy
	+ 5FU	5-5000 mg/m <sup>2</sup>
	+ Cisplatin	5-150 mg/m <sup>2</sup>
15	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ Oxaliplatin	5-200 mg/m <sup>2</sup>
	+ 5FU and optionally	5-5000 mg/m <sup>2</sup>
	+ Leucovorin	5-1000 mg/m <sup>2</sup>
20	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ paclitaxel	40-250 mg/m <sup>2</sup>
	+ CPT-11	4-400 mg/m <sup>2</sup>
25	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ paclitaxel	40-250 mg/m <sup>2</sup>
	+ 5-FU	5-5000 mg/m <sup>2</sup>
30	Compound of Formula I	0.1-100 mg/m <sup>2</sup>
	+ UFT	50-800 mg/m <sup>2</sup>
	+ CPT-11 and optionally	4-400 mg/m <sup>2</sup>
	+ leucovorin	5-1000 mg/m <sup>2</sup>

In the above Table I, "5FU" denotes 5-fluorouracil, "Leucovorin" can be employed as leucovorin calcium, "UFT" is a 1:4 molar ratio of tegafur:uracil, and As an example, "epothilone" is a compound described in WO 99/02514 or WO 00/50423, both of which are incorporated by reference herein in their entirety.

5           While Table I provides exemplary dosage ranges of the Formula I compounds and certain anticancer agents of the invention, when formulating the pharmaceutical compositions of the invention the clinician may utilize other dosages as warranted by the condition of the patient being treated. For example, when Compound 1 is administered in combination with carboplatin, Compound 1 may be administered at 5  
10   mg/m<sup>2</sup> to 60 mg/m<sup>2</sup> every 3 weeks and dosages for carboplatin may be within the range of 5 mg/m<sup>2</sup> to 1000 mg/m<sup>2</sup> or an AUC of 0.5-8 mg/ml x min. (dosage calculated per Calvert formula; Calvert *et al.*, J. Clin. Oncol., 1989, 7, 1748-1756), such as an AUC of 4-6 mg/ml x min.

          A compound of Formula I may be administered as a 10-minute to 3-hour  
15   infusion, for example, as a 60-minute infusion. The anti-proliferative agent may be administered as a 5-minute to 2-hour infusion, for example, as a 30-minute infusion.

          A compound of Formula I may be administered daily, weekly for 1-5 consecutive weeks, or every 2-5 weeks, for example, every 3 weeks. The compound of Formula I may also be administered in divided doses during each treatment cycle.  
20   For example, a compound of Formula I may be administered on day 1 and day 8 of the treatment cycle and repeated every 3 weeks.

          The anti-proliferative agent may be administered daily, weekly for 1-5 consecutive weeks, or every 2-5 weeks, for example, every 3 weeks. For example, the anti-proliferative agent may be administered on day 1 of the treatment cycle and  
25   repeated every 3 weeks. The anti-proliferative agent may also be administered in divided doses during each treatment cycle.

          A treatment cycle comprises that duration of time in which the desired doses (either single or split) of a compound of Formula I and an anti-proliferative agent are administered, before administration of these compounds is repeated in subsequent  
30   rounds of treatment. Thus, a patient may undergo several rounds of treatment, or treatment cycles, as determined necessary by the clinician or physician.

          In one embodiment of the present invention, Compound 1 may be

administered as a 60-minute infusion, 30-minutes prior to a 30-minute carboplatin infusion on day 1. This combination is administered every three weeks for at least one treatment cycle. A treatment cycle, which in this embodiment is 3 weeks in duration, comprises one round of chemotherapy using the compound of Formula I and the anti-proliferative agent. Treatment on this schedule may occur in, but is not limited to, four dose cycles. In another embodiment of the present invention, the dosage of Compound 1 is between 5 and 60 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 and 1000 mg/m<sup>2</sup> or between AUC 4 and 8. In yet another embodiment of the present invention, the dosage of Compound 1 is between 25 and 50 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 and 1000 mg/m<sup>2</sup> or between AUC 5 and 6.

As another example of the present invention, the doses of Compound 1 are split on days 1 and 8 with carboplatin being administered on day 1. This combination of agents is administered every three weeks for at least one treatment cycle. A treatment cycle in this case comprises administration of Compound 1 on days 1 and 8 with administration of carboplatin on day 1. This is followed by 2 weeks of no therapy where neither Compound 1 nor an anti-proliferative agent is administered. This 3-week period comprises one treatment cycle. Such treatment cycles are continued for as long as needed. In one embodiment of the present invention, the dosage of Compound 1 is between 5 and 60 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 and 1000 mg/m<sup>2</sup> or between AUC 4 and 8 (dosage calculated per Calvert formula; Calvert *et al.*, J. Clin. Oncol., 1989, 7, 1748-1756). In another embodiment of the present invention, the dosage of Compound 1 is between 15 and 30 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 and 1000 mg/m<sup>2</sup> or between AUC 5 and 6.

The actual dosage employed may be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small amounts until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired. Intermittent therapy (*e.g.*, one week out of three weeks or three out of four weeks) may also be used.

When employing the methods or compositions of the present invention, other agents used in the modulation of tumor growth or metastasis in a clinical setting, such as antiemetics, can also be administered as desired.

5 The combinations of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. Combinations of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a multiple combination formulation is inappropriate.

10 The chemotherapeutic agent(s) and/or radiation therapy can be administered according to therapeutic protocols well known in the art. It will be apparent to those skilled in the art that the administration of the chemotherapeutic agent(s) and/or radiation therapy can be varied depending on the disease being treated and the known effects of the chemotherapeutic agent(s) and/or radiation therapy on that disease. Also, in accordance with the knowledge of the skilled clinician, the therapeutic  
15 protocols (*e.g.*, dosage amounts and times of administration) can be varied in view of the observed effects of the administered therapeutic agents (*i.e.*, antineoplastic agent(s) or radiation) on the patient, and in view of the observed responses of the disease to the administered therapeutic agents.

The attending clinician or physician, in judging whether treatment is effective  
20 at the dosage administered, will consider the general well-being of the patient as well as more definite signs such as relief of disease-related symptoms, inhibition of tumor growth, actual shrinkage of the tumor, or inhibition of metastasis. Size of the tumor can be measured by standard methods such as radiological studies, *e.g.*, CAT or MRI scan, and successive measurements can be used to judge whether or not growth of the  
25 tumor has been retarded or even reversed. Relief of disease-related symptoms such as pain, and improvement in overall condition can also be used to help judge effectiveness of treatment.

In order to facilitate a further understanding of the invention, the following examples are presented primarily for the purpose of illustrating more specific details  
30 thereof. The scope of the invention should not be deemed limited by the examples, but to encompass the entire subject matter defined by the claims.

## EXAMPLES

### **Compounds:**

The following designations are used to identify the test compounds throughout the examples:

5

**Compound 1:** [1S-1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (compound of Formula Ia).

10 

### Chemicals and solutions:

Unless specified, chemicals and solutions used for the maintenance of cell culture were obtained from GIBCO/BRL. Sterile tissue culture ware was obtained from Corning, NY. All other reagents were from Sigma or Fisher at the highest grade available.

15 

### Drug Administration:

For administration of Compound 1 (an epothilone), two different excipients have been used: (1) ethanol/water (1:9, v/v) and (2) Cremophor<sup>®</sup>/ethanol/water (1:1:8, v/v). Compound 1 was first dissolved in ethanol or a mixture of Cremophor<sup>®</sup>/ethanol (50:50). Final dilution to the required dosage strength is made less than 1 h before drug administration. For parenteral administration (IV), dilution was made with water so that the dosing solutions contain the specified excipient composition described above.

20

## EXAMPLE 1

25 

### PHARMACOLOGICAL STUDIES OF COMPOUND 1 IN COMBINATION WITH CARBOPLATIN IN PATIENTS WITH ADVANCED CANCER

Compound 1 followed by carboplatin was given intravenously every 3 weeks to patients with advanced solid tumors treated with  $\leq 2$  prior chemotherapy regimens. Pharmacokinetics of both drugs were measured on the first course. Twenty-five (25) patients were treated at four dose levels – combining Compound 1 (30 or 40 mg/m<sup>2</sup>)

30



with 5-1000 mg/m<sup>2</sup> or AUC 5 or 6 (Calvert formula; Calvert *et al.*, J. Clin. Oncol., 1989, 7, 1748-1756) of carboplatin. A total of 91 courses were given. Transient CTC grade 4 neutropenia was noted at all dose levels and was dose limiting at 40 mg/m<sup>2</sup>/AUC 6 (2/2 patients with complicated grade 4 neutropenia). The major non-haematological toxicities were myalgia on day 4 and cumulative peripheral neuropathy. Seven patients withdrew from the trial because of neurotoxicity, grade 2/3 sensory neuropathy after at least 3 courses but grade 3 motor neuropathy in one patient on the first course. The regimen was active with 3/25 confirmed partial responses (breast, neuroendocrine and unknown primary carcinomas) and 12 patients have disease stabilization and clinical benefit from treatment.

The maximum tolerated dose of Compound 1 was 40 mg/m<sup>2</sup> with carboplatin AUC 5 (dosage calculated per Calvert formula, which allows determination of dosage based upon renal function of patient; Calvert *et al.*, J. Clin. Oncol., 1989, 7, 1748-1756).

## EXAMPLE 2

### PHARMACOLOGICAL STUDIES OF COMPOUND 1 IN COMBINATION WITH CARBOPLATIN IN PATIENTS WITH ADVANCED CANCER

Compound 1 was assessed in Phase I studies in combination with other chemotherapeutic agents. Compound 1 was given as a 60-minute infusion, 30 minutes prior to a 30-minute carboplatin infusion on day 1 every three weeks (or 21 days). Pharmacokinetic samples were taken during the first cycle. Twenty-five (25) patients were treated on this schedule at 4 dose levels (Compound 1 doses of 30 mg/m<sup>2</sup> or 40 mg/m<sup>2</sup> with carboplatin doses of 5-1000 mg/m<sup>2</sup>, or AUC 5 or 6). Dose limiting toxicity (DLT) occurred with 40 mg/m<sup>2</sup> of Compound 1 and carboplatin AUC 6 (per Calvert formula) on this schedule, with myelosuppression as the major toxicity.

A further 14 patients were recruited onto an amended schedule with Compound 1 doses being split and administered on days 1 and 8 every three weeks (or 21 days) and carboplatin being administered on day 1. DLT on this schedule was observed with carboplatin AUC 6 and with Compound 1 at 25 mg/m<sup>2</sup> being administered on days 1 and 8. The preceding dose level (carboplatin AUC 6 and

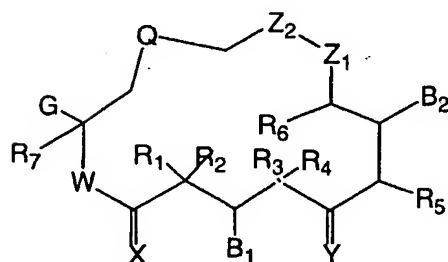
Compound 1 at 20 mg/m<sup>2</sup> on days 1 and 8) was expanded and a total of 8 patients were treated.

5       Thirty-nine (39) patients (18 male/19 female/2 missing) were treated over seven (7) dose level; 92% were WHO performance status 0 or 1 at entry; mean age 55 years (range 31-74). Fifty-six percent (56%) of patients had received prior chemotherapy (3 or fewer regimens) and 51% had prior radiotherapy. Major toxicities were myelosuppression, myalgia and peripheral neuropathy. Eleven patients withdrew from the study because of study drug toxicity, with peripheral neuropathy being reported in all these patients.

10       The regimen was active: partial responses were reported in five (5) patients (2 breast, neuro-endocrine, unknown primary carcinomas and mesothelioma), 48% of patients showed disease stabilization for more than two months. Analysis of Compound 1 plasma levels show that C<sub>max</sub> and AUC increase with dose, the volume of distribution is high and half-life is ~30 hours. Thus, Compound 1 can safely be  
15       combined with carboplatin, with the major toxicities being neutropenia and peripheral neuropathy. Dividing the dose of Compound 1 allows a higher dose intensity to be achieved.

**What Is Claimed Is:**

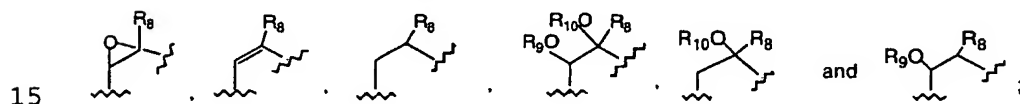
1. A method for the treatment of a proliferative disease which comprises administering to a mammalian specie in need thereof a therapeutically effective amount of (1) a compound of formula I and (2) at least one anti-proliferative agent,



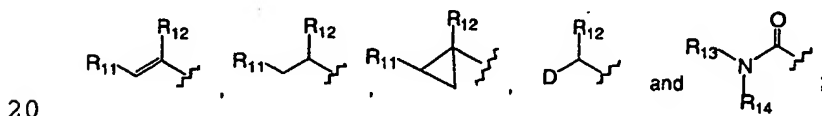
I

wherein:

Q is selected from the group consisting of



G is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heterocyclo,



W is O or N R<sub>15</sub>;

X is O or H, H;

Y is selected from the group consisting of O; H, OR<sub>16</sub>; OR<sub>17</sub>, OR<sub>17</sub>; NOR<sub>18</sub>; H, NHOR<sub>19</sub>; H, NR<sub>20</sub>R<sub>21</sub>; H, H; and CHR<sub>22</sub>; wherein OR<sub>17</sub>, OR<sub>17</sub> can be a cyclic ketal;

Z<sub>1</sub> and Z<sub>2</sub> are independently selected from the group consisting of CH<sub>2</sub>, O,

NR<sub>23</sub>, S, and SO<sub>2</sub>, wherein only one of Z<sub>1</sub> and Z<sub>2</sub> can be a heteroatom;

B<sub>1</sub> and B<sub>2</sub> are independently selected from the group consisting of OR<sub>24</sub>, OCOR<sub>25</sub>, and O-C(=O)-NR<sub>26</sub>R<sub>27</sub>, and when B<sub>1</sub> is H and Y is OH, H, they can form a six-membered ring ketal or acetal;

5 D is selected from the group consisting of NR<sub>28</sub>R<sub>29</sub>, NR<sub>30</sub>COR<sub>31</sub> and saturated heterocycle;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>13</sub>, R<sub>14</sub>, R<sub>18</sub>, R<sub>19</sub>, R<sub>20</sub>, R<sub>21</sub>, R<sub>22</sub>, R<sub>26</sub> and R<sub>27</sub> are independently selected from the group consisting of H, alkyl, substituted alkyl, and aryl, and when R<sub>1</sub> and R<sub>2</sub> are alkyl can be joined to form a cycloalkyl, and when R<sub>3</sub>  
10 and R<sub>4</sub> are alkyl can be joined to form a cycloalkyl;

R<sub>9</sub>, R<sub>10</sub>, R<sub>16</sub>, R<sub>17</sub>, R<sub>24</sub>, R<sub>25</sub> and R<sub>31</sub> are independently selected from the group consisting of H, alkyl, and substituted alkyl;

R<sub>8</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>28</sub>, R<sub>30</sub>, R<sub>32</sub>, and R<sub>33</sub> are independently selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl and  
15 heterocycle;

R<sub>15</sub>, R<sub>23</sub> and R<sub>29</sub> are independently selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocycle, R<sub>32</sub>C=O, R<sub>33</sub>SO<sub>2</sub>, hydroxy, O-alkyl or O-substituted alkyl; and pharmaceutically acceptable salts thereof and any hydrates, solvates or geometric, optical and stereoisomers thereof,  
20 wherein said compound of formula I and said anti-proliferative agent are administered simultaneously or sequentially.

2. The method of claim 1 wherein the antiproliferative agent is administered after administration of the compound of formula I.

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3. The method of claim 2 for the treatment of cancerous solid tumors.

4. The method of claim 2 for the treatment of refractory tumors.

30 5. The method of claim 1 wherein the anti-proliferative agent is selected from the group consisting of a microtubule affecting agent, an alkylating agent, an anti-metabolite, epidophyllotoxin, an antineoplastic enzyme, a topoisomerase

inhibitor, procarbazine, mitoxantrone, inhibitors of cell cycle progression, radiation and a platinum coordination complex.

- 5
6. The method of claim 5 wherein the anti-proliferative agent is carboplatin.
7. The method of claim 1 wherein the compound of formula I is [1S-1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1).
- 10
8. The method of claim 2 wherein the compound of formula I is [1S-1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1).
- 15
9. The method of claim 1 wherein the compound of formula I is [1S-1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) and the anti-proliferative agent is carboplatin.
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10. The method of claim 2 wherein the compound of formula I is [1S-1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) and the anti-proliferative agent is carboplatin.
- 25
11. A method for modulating tumor growth in a human in need thereof, comprising sequential or simultaneous administration of [1S-1R\*,3R\*(E),7R\*,10S\*,11R\*,12R\*,16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) and carboplatin.
- 30

12. The method of claim 11 wherein said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is administered first, followed by administration of carboplatin, and wherein this combination of Compound 1 and carboplatin is administered every three weeks for at least one treatment cycle.
13. The method of claim 12 wherein said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is administered as a 60-minute infusion, 30 minutes prior to a 30-minute infusion of carboplatin.
14. The method of claim 12 wherein the dosage of said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is between 5 mg/m<sup>2</sup> and 60 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 mg/m<sup>2</sup> and 1000 mg/m<sup>2</sup>.
15. The method of claim 14 wherein the dosage of said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is between 25 mg/m<sup>2</sup> and 50 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 mg/m<sup>2</sup> and 1000 mg/m<sup>2</sup>.
16. The method of claim 11 wherein said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is administered on day 1 and again on day 8, and carboplatin is administered on day 1, for at least one treatment cycle.

17. The method of claim 16 wherein said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is administered as a 60-minute infusion, 30 minutes prior to a 30-minute infusion of carboplatin.
18. The method of claim 16 wherein the dosage of said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is between 5 mg/m<sup>2</sup> and 60 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 mg/m<sup>2</sup> and 1000 mg/m<sup>2</sup>.
19. The method of claim 18 wherein the dosage of said [1S 1R\*, 3R\*(E), 7R\*, 10S\*, 11R\*, 12R\*, 16S\*]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (Compound 1) is between 15 and 30 mg/m<sup>2</sup> and the dosage of carboplatin is between 5 mg/m<sup>2</sup> and 1000 mg/m<sup>2</sup>.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/18733

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61K 31/33, 31/335, 31/425

US CL : 514/183, 366, 475

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/183, 366, 475

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,365,749 B1 (KIM et al.) 02 April 2002 (02.04.02), see the entire document.	1-19
A	US 6,399,638 B1 (VITE et al.) 04 June 2002 (04.06.02), see the entire document.	1-19

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "&" document member of the same patent family

Date of the actual completion of the international search

10 September 2003 (10.09.2003)

Date of mailing of the international search report

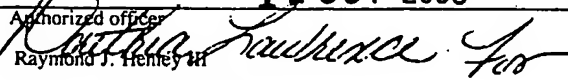
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